#14
3/26/02
PB

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Schubert et al.

Serial No.: 09/372,036
Filed June, 06, 1993
For: PROCESS AND AGENTS FOR DETECTING LISTERIAS
Group Art Unit: 1645
Examiner: Baskar, P.

DECLARATION

Honorable Commissioner of
Patents and Trademarks
Washington, D.C., 200231

MADAM/SIR:

The Declarant, Andreas Bubert, being duly warned, declares and says:

THAT he is a German citizen, residing at 64401 Gross-Bieberau, Germany;

THAT he is a biologist having studied at the University of Würzburg, Germany, from 1981 to 1988;

THAT he graduated from the University of Würzburg in 1988,

THAT he obtained the Dr. rer. nat. degree in the field of microbiology and biochemistry from the University of Würzburg in 1993;

THAT he worked as a postdoctoral research associate at the University of Würzburg from 1993 to 1998;

THAT, in 1998 he joined the Research and Development Department of the Scientific Laboratory Products Division of MERCK KGaA, Darmstadt, Germany;

THAT, since 1992 he has been working in the fields of immunology and immunologic testing, including Listeria and Salmonella;

THAT he is author or co-author of numerous articles in the field of immunology and immunologic testing;

THAT he is inventor or co-inventor of numerous inventions in the field of immunology and immunologic testing;

THAT he is familiar with issues related to immunology and immunologic testing, especially with the subject invention disclosed and claimed in U.S. Patent Application Ser. No. 09/372,036, filed June 06, 1993, by P. Schubert et al (hereinafter referred to as Application), of which he is co-inventor;

THAT the procedure according to Kohler does not result in one single kind of antibody, but in a mixture containing a plurality of different antibodies with different specificities. The antiserum raised against purified p60 according to Kohler may also contain antibodies that bind an epitope from the peptides according to SEQ. ID. NO: 17, 20, 26, 29, 30 or 31.

THAT even though the antibodies specific for the claimed epitopes might be present in the mixture produced according to Kohler, it is not possible to isolate them without having the information presented in the present invention. If one does not know to which epitope an antibody within a mixture of different antibodies binds, one is not able to reproducibly identify or isolate it.

THAT the teaching of Lerner et al. that peptides derived from the sequence of the whole protein can be used to raise antibodies is not generally applicable. The view of Lerner is also disapproved in literature (see declaration of S. Neumann, April, 1994).

THAT only very few peptides out of numerous peptides whose sequence is specific for L. monocytogenes are suitable for the preparation of specific antibodies. Certainly a person skilled in the art would not have had a reasonable expectation of success of generating antibodies having the claimed specificities.

THAT no hint is given in any document of prior art that the peptides claimed in the present invention are suitable for the generation of antibodies specific for L. monolcytogenes.

THAT the data shown in Appendix 1 demonstrate that out of a group of several peptides which could be selected for the generation of antibodies only a few peptides are successful for generating specific antibodies.

THAT the undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the APPLICATION or any patent issuing thereon.

Done, this November 08, 2001, at Darmstadt, Germany

Bubert

Dr. Andreas Bubert

APPENDIX 1

1. Production of Antibodies

The peptides A-H have been prepared essentially as described in Example 9 of the present application.

The peptides were conjugated with glucose dehydrogenase from Bacillus megaterium. The conjugates were used for immunisations.

Antisera against the conjugates containing the peptides were produced essentially as described in Example 11 of the present application.

Peptide	Name in sequence listing	Resemblance to SEQ ID Nr.	Peptide Sequence
A	Pep D/A4B6	[20]	C-QQQTAPKAPTE
B	A2	[17]	C-STPVAPTQEVKK
C	Mono1-Pep		C-QVNNEVAAAEKTEK
D	Mono3-Pep		C-KLAIKQTANTATPK
E	52-8		C-ETKETPVVDQN
F	52-9		C-PKVAETKETP
G			C-QQAAPAAETK
H			C-TNTNTNNTNTN

The position of the sequence of the peptides A-H within the p60 sequence is shown in the attached sequence listing.

2. Analysis of the Antibodies

The antisera raised against the peptides were analysed both with the peptide used for imunisation (positive control) and with proteins from culture supernatants of *L. monocytogenes* (containing the p60 protein).

Peptide	Immunisation In:	Recognition: Peptide (Pos. Ctrl)	Recognition: p60 by Immunoblot	Specific for L. mono. p60
A	RABBIT	YES	YES	YES
B	RABBIT	YES	YES	YES
C	RABBIT	YES	NO	NO
D	RABBIT	YES	NO	NO
E	RABBIT	YES	YES	NO
F	RABBIT	YES	YES	NO
G	RABBIT	YES	YES	NO
H	RABBIT	YES	YES	NO

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1437 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Listeria monocytogenes

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..1437

(xi) SEQUENCE DESCRIPTION:

```
                                                  GT CGA                    IIa; [9]

ATG AAT ATG AAA AAA GCA ACT ATC GCG GCT ACA GCT GGG ATT GCG GTA      48
CTG AAT ATG AAA AAA GCA AC                                          IIa; [9]
    AAT ATG AAA AAA GC                                              Ia; [1]
 X1 AAT ATG AAA AAA GC X2                                             Va
                                 GCT ACA GCT GGG ATT GCG GT         IIc; [11]
Met Asn Met Lys Lys Ala Thr Ile Ala Ala Thr Ala Gly Ile Ala Val
  1               5                  10                  15

ACA GCA TTT GCT GCT CCA ACA ATC GCA TCC GCA AGC ACT GTA GTA GTC      96
Thr Ala Phe Ala Ala Pro Thr Ile Ala Ser Ala Ser Thr Val Val Val
            20                  25                  30

GAA GCT GGT GAT ACT CTT TGG GGT ATC GCA CAA AGT AAA GGG ACT ACT     144
Glu Ala Gly Asp Thr Leu Trp Gly Ile Ala Gln Ser Lys Gly Thr Thr
        35                  40                  45

GTT GAC GCA ATT AAA AAA GCA AAC AAT TTA ACA ACA GAT AAA ATC GTA     192
Val Asp Ala Ile Lys Lys Ala Asn Asn Leu Thr Thr Asp Lys Ile Val
    50                  55                  60
```

Listeria iap-/p60

```
CCA GGT CAA AAA TTA CAA GTA AAT AAT GAG GTT GCT GCT GCT GAA AAA      240
                     TA ACT GAG GTA GCG AGC GAA                    IIf;[14]
                        CT GAG GTA GCG AGC                         If;[6]
                      X1 CT GAG GTA GCG AGC X2                     Vf
Pro Gly Gln Lys Leu Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys
 65                  70                  75                  80
                    Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys    (3);[39]
                    Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys    (2g);[32]
                        Val Asn Asn Glu Val Ala Ala Ala Glu Lys    IIIe;[21]
                     X3 Val Asn Asn Glu Val Ala Ala Ala Glu Lys        IVe
                    Gln Val Asn Asn Glu Val Ala Ala Ala Glu Lys    Mono1-Pep

ACA GAG AAA TCT GTT AGC GCA ACT TGG TTA AAC GTC CGT ACT GGC GCT      288
Thr Glu Lys Ser Val Ser Ala Thr Trp Leu Asn Val Arg Thr Gly Ala
                 85                  90                  95
Thr Glu Lys Ser Val Ser Ala Thr Trp Leu Asn Val Arg Thr Gly Ala    (3); [39]
Thr Glu Lys                                                        (2g);[32]
Thr Glu                                                            IIIe;[21]
Thr Glu X4                                                         IVe
Thr Glu Lys                                                        Mono1-Pep

GGT GTT GAT AAC AGT ATT ATT ACG TCC ATC AAA GGT GGA ACA AAA GTA      336
Gly Val Asp Asn Ser Ile Ile Thr Ser Ile Lys Gly Gly Thr Lys Val
            100                 105                 110
Gly Val Asp Asn Ser Ile Ile Thr Ser Ile Lys Gly Gly Thr Lys Val     3); [39]

ACT GTT GAA ACA ACC GAA TCT AAC GGC TGG CAC AAA ATT ACT TAC AAC      384
Thr Val Glu Thr Thr Glu Ser Asn Gly Trp His Lys Ile Thr Tyr Asn
        115                 120                 125
Thr Val Glu Thr Thr Glu Ser Asn Gly Trp His Lys Ile Thr Tyr Asn     3); [39]

GAT GGA AAA ACT GGT TTC GTT AAC GGT AAA TAC TTA ACT GAC AAA GCA      432
Asp Gly Lys Thr Gly Phe Val Asn Gly Lys Tyr Leu Thr Asp Lys Ala
    130                 135                 140
Asp Gly Lys Thr Gly Phe Val Asn Gly Lys Tyr Leu Thr Asp Lys Ala    (3); [39]

GTA AGC ACT CCA GTT GCA CCA ACA CAA GAA GTG AAA AAA GAA ACT ACT      480
ACT AGC ACT CCA GTT GGT TAA AC                                     IIg;[15]
    AGC ACT CCA GTT GGT TA                                         Ig;[7]
 X1 AGC ACT CCA GTT GGT TA X2                                      Vg
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys Glu Thr Thr
145                 150                 155                 160
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys Glu Thr Thr    (3); [39]
Val Ser Thr Pro Val Ala Pro Thr Gln                                (2a);[26]
Val Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys                (2e);[30]
            Pro Val Ala Pro Thr Gln Glu Val Lys Lys                (2f);[31]
            Pro Val Ala Pro Thr Gln                                IIIa;[17]
         X3 Pro Val Ala Pro Thr Gln X4                             IVa
    Ser Thr Pro Val Ala Pro Thr Gln Glu Val Lys Lys                A2
```

```
ACT CAA CAA GCT GCA CCT GTT GCA GAA ACA AAA ACT GAA GTA AAA CAA        528
Thr Gln Gln Ala Ala Pro Val Ala Glu Thr Lys Thr Glu Val Lys Gln
                    165                 170                 175
Thr Gln Gln Ala Ala Pro Val Ala Glu Thr Lys Thr Glu Val Lys Gln  (3); [39]
                                                Glu Val Lys Gln  (2h);[33]
    Gln Gln Ala Ala Pro Ala Ala Glu Thr Lys                      52-10

ACT ACA CAA GCA ACT ACA CCT GCG CCT AAA GTA GCA GAA ACG AAA GAA        576
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala Glu Thr Lys Glu
            180                 185                 190
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala Glu Thr Lys Glu  (3); [39]
Thr Thr Gln Ala Thr Thr Pro Ala                                  (2h);[33]
Thr Thr Gln Ala Thr Thr Pro Ala Pro Lys Val Ala                  (2b);[27]
    Thr Gln Ala Thr Thr Pro Ala                                  IIIb;[18]
 X3 Thr Gln Ala Thr Thr Pro Ala X4                               IVb
                                Pro Lys Val Ala Glu Thr Lys Glu      52-9
                                                Glu Thr Lys Glu      52-8

ACT CCA GTA ATA GAT CAA AAT GCT ACT ACA CAC GCT GTC AAA AGC GGT        624
Thr Pro Val Ile Asp Gln Asn Ala Thr Thr His Ala Val Lys Ser Gly
        195                 200                 205
Thr Pro Val Ile Asp Gln Asn Ala Thr Thr His Ala Val Lys Ser Gly  (3); [39]
Thr Pro                                                          52-9
Thr Pro Val Val Asp Gln Asn                                      52-8

GAC ACT ATT TGG GCT TTA TCC GTA AAA TAC GGT GTT TCT GTT CAA GAC        672
Asp Thr Ile Trp Ala Leu Ser Val Lys Tyr Gly Val Ser Val Gln Asp
    210                 215                 220
Asp Thr Ile Trp Ala Leu Ser Val Lys Tyr Gly Val Ser Val Gln Asp  (3); [39]

ATT ATG TCA TGG AAT AAT TTA TCT TCT TCT TCA ATT TAT GTA GGT CAA        720
Ile Met Ser Trp Asn Asn Leu Ser Ser Ser Ser Ile Tyr Val Gly Gln
225                 230                 235                 240
Ile Met Ser Trp Asn Asn Leu Ser Ser Ser Ser Ile Tyr Val Gly Gln  (3); [39]

AAG CTT GCT ATT AAA CAA ACT GCT AAC ACA GCT ACT CCA AAA GCA GAA        768
                    CAA ACT GCT AAC ACA GCT ACT                   IId;[12]
                        ACT GCT AAC ACA GCT                       Id;[4]
                     X1 ACT GCT AAC ACA GCT X2                    Vd
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys Ala Glu
                    245                 250                 255
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys Ala Glu  (3); [39]
    Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr                  (2c);[28]
        Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys          (2i);[34]
        Ala Ile Lys Gln Thr Ala Asn Thr Ala                      IIIc;[19]
     X3 Ala Ile Lys Gln Thr Ala Asn Thr Ala X4                   IVc
Lys Leu Ala Ile Lys Gln Thr Ala Asn Thr Ala Thr Pro Lys          Mono3-Pep

GTG AAA ACG GAA GCT CCA GCA GCT GAA AAA CAA GCA GCT CCA GTA GTT        816
Val Lys Thr Glu Ala Pro Ala Ala Glu Lys Gln Ala Ala Pro Val Val
```

```
                260                 265                 270
Val Lys Thr Glu Ala Pro Ala Ala Glu Lys Gln Ala Ala Pro Val Val   (3); [39]

AAA GAA AAT ACT AAC ACA AAT ACT GCT ACT ACA GAG AAA AAA GAA ACA        864
Lys Glu Asn Thr Asn Thr Asn Thr Ala Thr Thr Glu Lys Lys Glu Thr
        275                 280                 285
Lys Glu Asn Thr Asn Thr Asn Thr Ala Thr Thr Glu Lys Lys Glu Thr   (3); [39]

GCA ACG CAA CAA CAA ACA GCT CCT AAA GCA CCA ACA GAA GCT GCA AAA        912
Ala Thr Gln Gln Gln Thr Ala Pro Lys Ala Pro Thr Glu Ala Ala Lys
    290                 295                 300
Ala Thr Gln Gln Gln Thr Ala Pro Lys Ala Pro Thr Glu                (3); [39]
        Gln Gln Gln Thr Ala Pro Lys Ala Pro Thr Glu                (2d);[29]
            Gln Gln Thr Ala Pro Lys Ala Pro Thr                    IIId;[20]
         X3 Gln Gln Thr Ala Pro Lys Ala Pro Thr X4                 IVd
            Gln Gln Thr Thr Thr Lys Ala Pro Thr                    IIIi;[25]
         X3 Gln Gln Thr Thr Thr Lys Ala Pro Thr X4                 IVi
        Glu Gln Gln Thr Thr Thr Lys Ala Pro Thr Gln                (5d);[38]
        Gln Gln Gln Thr Ala Pro Lys Ala Pro Thr Glu                A4B6

CCA GCT CCT GCA CCA TCT ACA AAC ACA AAT GCT AAT AAA ACG AAT ACA        960
Pro Ala Pro Ala Pro Ser Thr Asn Thr Asn Ala Asn Lys Thr Asn Thr
305                 310                 315                 320
                                                            Thr    56-9

AAT ACA AAT ACA AAC AAT ACT AAT ACA CCA TCT AAA AAT ACT AAT ACA       1008
Asn Thr Asn Thr Asn Asn Thr Asn Thr Pro Ser Lys Asn Thr Asn Thr
                325                 330                 335
Asn Thr Asn Thr Asn Asn Thr Asn Thr Asn                        56-9

AAC TCA AAT ACT AAT ACG AAT ACA AAC TCA AAT ACG AAT GCT AAT CAA       1056
Asn Ser Asn Thr Asn Thr Asn Thr Asn Ser Asn Thr Asn Ala Asn Gln
            340                 345                 350

GGT TCT TCC AAC AAT AAC AGC AAT TCA AGT GCA AGT GCT ATT ATT GCT       1104
              C AAT AAC AGC AAT TCA AGT GC                         IIe;[13]
                AT AAC AGC AAT TCA AG                              Ie;[5]
              X1 AT AAC AGC AAT TCA AG X2                          Ve
Gly Ser Ser Asn Asn Asn Ser Asn Ser Ser Ala Ser Ala Ile Ile Ala
        355                 360                 365

GAA GCT CAA AAA CAC CTT GGA AAA GCT TAT TCA TGG GGT GGT AAC GGA       1152
Glu Ala Gln Lys His Leu Gly Lys Ala Tyr Ser Trp Gly Gly Asn Gly
    370                 375                 380

CCA ACT ACA TTT GAT TGC TCT GGT TAC ACT AAA TAT GTA TTT GCT AAA       1200
Pro Thr Thr Phe Asp Cys Ser Gly Tyr Thr Lys Tyr Val Phe Ala Lys
385                 390                 395                 400
```

```
GCG GGT ATC TCC CTT CCA CGT ACA TCT GGC GCA CAA TAT GCT AGC ACT        1248
Ala Gly Ile Ser Leu Pro Arg Thr Ser Gly Ala Gln Tyr Ala Ser Thr
                405                 410                 415

ACA AGA ATT TCT GAA TCT CAA GCA AAA CCT GGT GAT TTA GTA TTC TTC        1296
Thr Arg Ile Ser Glu Ser Gln Ala Lys Pro Gly Asp Leu Val Phe Phe
            420                 425                 430

GAC TAT GGT AGC GGA ATT TCT CAC GTT GGT ATT TAT GTT GGT AAT GGT        1344
Asp Tyr Gly Ser Gly Ile Ser His Val Gly Ile Tyr Val Gly Asn Gly
        435                 440                 445

CAA ATG ATT AAC GCG CAA GAC AAT GGC GTT AAA TAC GAT AAC ATC CAC        1392
Gln Met Ile Asn Ala Gln Asp Asn Gly Val Lys Tyr Asp Asn Ile His
    450                 455                 460

GGC TCT GGC TGG GGT AAA TAT CTA GTT GGC TTC GGT CGC GTA TAA            1437
                        TT GGC TTC GGT CGC GTA GAA TTC ATA
                                                        IIb;[10]
                           GC TTC GGT CGC GTA           Ib; [21
                        X1 GC TTC GGT CGC GTA X2        Vb
Gly Ser Gly Trp Gly Lys Tyr Leu Val Gly Phe Gly Arg Val  *
465                 470                 475
```